

Patent
Application No. 09/843,150
Atty. Dkt. No. 065691/0219

Detailed and Complete Listing of Claims:

1. (Currently Amended) An isolated DNA molecule comprising at least a sequence A flanked by at least site specific recombinase targeting sequences (SSRTS) L1, and at least a sequence B flanked by at least site specific recombinase targeting sequences (SSRTS) L2, said SSRTS L1 and SSRTS L2 being unable to recombine with one another, wherein:

(i) sequences L1 are in an orientation opposite to one another, wherein said sequences point towards each other or point away from each other,

(ii) sequences L2 are in an orientation opposite to one another, wherein said sequences point towards each other or point away from each other, and

(iii) the order of SSRTS sequences in said DNA molecule is 5'-L1-L2-L1-L2-3' 5'-L1-L2-sequence A-sequence B-L1-L2-3'.

2-4. (Canceled).

5. (Previously Presented) The DNA molecule according to claim 1, wherein sequences A and B are in a direction opposite to each other.

6. (Previously Presented) The DNA molecule according to claim 1, wherein the recombinase specific of said SSRTS L1 and the recombinase specific of said SSRTS L2 are the same.

7. (Canceled).

8. (Previously Presented) The DNA molecule according to claim 6, wherein said recombinase specific of said SSRTS is selected from the group consisting of Cre recombinase of bacteriophage P1, the FLP recombinase of *Saccharomyces cerevisiae*, the R recombinase of *Zygosaccharomyces rouxii* pSR1, the A recombinase of *Kluyveromyces drosophilarius*

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pKD1, the A recombinase of *Kluyveromyces waltii* pKW1, the integrase λ Int, the recombinase of the GIN recombination system of the Mu phage, and bacterial β recombinase.

9. (Previously Presented) The DNA molecule according to claim 8, wherein said recombinase is said Cre recombinase of bacteriophage P1.

10. (Previously Presented) The DNA molecule according to claim 9, wherein said SSRTSL1 and/or L2 specific for said Cre recombinase are selected from the group consisting of Lox P1, Lox 66, Lox 71, Lox 511, Lox 512, Lox 514, and mutated Lox P1 sequences, wherein said mutated Lox P1 sequences comprise at least one point mutation in the spacer sequence.

11. (Previously Presented) The DNA molecule according to claim 10, wherein either SSRTS L1 comprises the Lox P1 nucleotide sequence (SEQ ID NO. 1) and SSRTS L2 comprises the Lox 511 nucleotide sequence (SEQ ID NO. 2) or SSRTS L1 comprises the Lox 511 sequence and SSRTS L2 comprises Lox P1 sequence.

12. (Previously Presented) The DNA molecule according to claim 8, wherein the recombinase is the FLP recombinase of *Saccharomyces cerevisiae*.

13. (Previously Presented) The DNA molecule according to claim 12, wherein said SSRTS L1 and/or L2 specific for said FLP recombinase are chosen from the group consisting of the sequences FRT-S and FRT-F3^{0.88}.

14. (Canceled).

15. (Previously Presented) The DNA molecule according to claim 1, wherein said sequences A and B are selected from the group consisting of non transcribed sequence, transcribed but not translated sequence, and transcribed and translated sequence.

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16. (Previously Presented) The DNA molecule according to claim 15, wherein at least the sequences A and/or B are transcribed and translated, wherein said translated sequences code for at least one protein.

17. (Previously Presented) The DNA molecule according to claim 16, wherein said protein is a protein of interest.

18. (Previously Presented) The DNA molecule according to claim 16, wherein sequences A and/or B encode at least one exon, or a fragment thereof.

19. (Original) The DNA molecule according to claim 18, wherein said exon differs from the wild type exon of a protein of interest by one or more point mutations.

20. (Previously Presented) The DNA molecule according to claim 16, wherein said protein is encoded by a cDNA sequence, and wherein an IRES sequence is inserted 5', or 3', or 5' and 3' to said cDNA sequence.

21. (Previously Presented) The DNA molecule according to claim 17, wherein said reporter protein is selected in the group consisting of autofluorescent proteins and enzymes detectable by a histochemical process.

22. (Previously Presented) The DNA molecule according to claim 21, wherein said autofluorescent protein is selected from the group consisting of the green fluorescent protein (GFP), the enhanced green fluorescent protein (EGFP), the red fluorescent protein (RFP), the blue fluorescent protein (BFP), and the yellow fluorescent protein (YFP).

23. (Previously Presented) The DNA molecule according to claim 21, wherein said enzyme, detectable by a histochemical process, is selected in the group consisting of β -galactosidase, β -glucuronidase, alkaline phosphatase, luciferase, alcohol dehydrogenase, chloramphenicol acetyl transferase.

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24. (Previously Presented) A vector comprising the isolated DNA molecule of claim 1.
25. (Canceled).
26. (Withdrawn) An isolated transgenic host cell transformed by an isolated DNA molecule according to claim 1.
27. (Withdrawn) The isolated transgenic host cell according to claim 26 wherein sequences of homology are present at both extremities of said DNA molecule.
28. (Withdrawn) The isolated transgenic host cell according to claim 27 wherein said isolated DNA molecule or said vector is integrated by homologous recombination in at least one targeted locus of the genome of said cell.
29. (Withdrawn) The isolated transgenic host cell according to claim 26 wherein said isolated DNA molecule or said vector is integrated in sites of the genome chosen among polyA sites and gene promoters.
30. (Withdrawn) The isolated transgenic host cell according to claim 26 wherein said isolated DNA molecule or said vector is randomly integrated in at least one locus of the genome of said cell.
31. (Withdrawn) The isolated transgenic host cell according to claim 26 wherein said isolated DNA molecule or said vector is maintained in said cell in an episomal form.
32. (Withdrawn) A transgenic non-human organism comprising at least one cell according to claim 26.

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33. (Withdrawn) A method for the stable inversion of a DNA sequence comprising:

(i) contacting a DNA molecule according to claim 1 with at least one site specific recombinase targeting sequence SSRTS L1 and one site specific recombinase targeting sequence SSRTS L2;

(ii) inversion of said sequences A and B or sequence A or sequence B by recombination catalyzed by said recombinase at either SSRTS L1 or L2 sequences; and

(iii) excision by recombination catalyzed by said recombinase of a DNA fragment comprised in between the SSRTS L1 or L2 sequences that are now present in direct orientation following the inversion of step (ii), and that are able to recombine with one another.

34. (Withdrawn) The method according to claim 33 wherein said DNA fragment excised in step (iii) comprises the sequence A.

35. (Withdrawn) A method for obtaining a transgenic cell of which at least one allele of a DNA sequence of interest is invalidated by a process of conditional deletion and the genome of which comprises a gene selected among reporter gene, market gene and gene encoding a protein of interest, inserted at the place of the DNA fragment deleted by said process of conditional deletion, said method comprises the steps of:

(i) preparation of a DNA molecule according to claim 1 wherein sequence A or sequence B is coding at least for part of the DNA fragment of interest to be invalidated and sequence B or sequence A is coding at least for a reporter gene;

(ii) obtention of a transgenic cell genetically modified by the targeted insertion by homologous recombination at the place of said DNA sequence of interest, of a DNA molecule prepared at step (i);

(iii) contacting said DNA molecule with at least one site specific recombinase targeting sequence SSRTS L1 and one site specific recombinase targeting sequence SSRTS L2;

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(iv) inversion of sequences A and B or sequence A or sequence B by recombination catalyzed by said recombinase at either SSRTS L1 or SSRTS L2 sequences; and

(v) excision of a DNA sequence by recombination catalyzed by said recombinase at SSRTS L2 or SSRTS L1 respectively, these SSRTS L2 or SSRTS L1 sequences being now present in direct orientation following to the inversion of step (iii), and being to recombine with one another.

36. (Withdrawn) The method of claim 35, wherein the order of sequences in said DNA molecule is 5'-L1-sequence A-L2-sequence B-L1-L2-3' and wherein a sequence of homology with the DNA sequence of interest are present at both extremities of said DNA molecule and wherein, the DNA fragment excised in step (v) comprises sequence A.

37. (Withdrawn) The method of claim 35, wherein the order of sequences in said DNA molecule is 5'-L1-L2- sequence A-sequence B-L1-L2-3' and wherein a sequence of homology with the DNA sequence of interest are present at both extremities of said DNA molecule.

38. (Withdrawn) The method of claim 35 wherein the order of sequences in said DNA molecule is 5'-L1-L2-sequence A-L1-sequence B-L2-3' and wherein a sequence of homologs with the DNA sequence of interest are present at both extremities of said DNA molecule.

39. (Withdrawn) A method to perform site-specific recombination mediated cassette exchange (RMCE), said method comprising:

(i) preparation of a first DNA molecule comprising a first DNA sequence of interest flanked by incompatible site specific recombinase targeting sequences SSRTS L1 and L2 in an opposite direction, obtainable by the method of claim 33;

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(ii) preparation of a second DNA molecule comprising a second DNA sequence of interest flanked by the same incompatible SSRTS L1 and L2 as in step (i) in an opposite direction, by *in vitro* DNA cloning;

(iii) contacting said first and said second DNA molecule with at least one recombinase targeting sequence SSRTS L1 and one recombinase specific to said SSRTS L2;

(iv) exchange by recombination catalyzed by said recombinase of said first and said second DNA sequence of interest comprised in between the SSRTS L1 and L2.

40. (Withdrawn) The method according to claim 39 wherein said second DNA molecule of step (ii) is obtainable by the method of claim 33.

41. (Withdrawn) The method according to claim 33 wherein the steps are made in a cell free system.

42. (Withdrawn) The method according to claim 33 wherein the steps are made in an isolated transgenic host cell transformed by an isolated DNA molecule comprising at least a sequence A flanked by at least site specific recombinase targeting sequences (SSRTS) L1, and at least a sequence B flanked by at least site specific recombinase targeting sequences (SSRTS) L2, said SSRTS L1 and SSRTS L2 being unable to recombine with one another, and wherein:

- (i) sequences L1 are in opposite orientation, and
- (ii) sequences L2 are in opposite orientation, and
- (iii) the order of SSRTS sequences in said DNA molecule is 5'-L1-L2-L1-L2-3'.

43. (Withdrawn) The method according to claim 42, further comprising introducing into the cell a gene encoding the corresponding site-specific recombinase.

44. (Withdrawn) The method according to claim 43, wherein the gene encoding said site-specific recombinase is contained in an expression vector.

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45. (Withdrawn) The method according to claim 43, wherein the gene encoding said site-specific recombinase is stably inserted into the genome of said cell.

46. (Withdrawn) The method according to claim 33, wherein either SSRTS L1 comprises the Lox P1 sequence and SSRTS L2 comprises the Lox 511 sequence, or SSRTS L1 comprises the Lox 511 sequence and SSRTS L2 comprises Lox P1 sequence, and wherein the corresponding site-specific recombinase is Cre or its material or synthetic variants.

47-48. (Canceled).

49. (Withdrawn) A non-human living organism that comprises at least one transgenic cell obtainable by the method of claim 33.

50. (Withdrawn) The non-human living organism of claim 49, wherein said organism is selected from the group consisting of bacteria, yeast, *Caenorhabditis elegans*, *Drosophila melanogaster*, zebrafish, mice, rat, rabbit, hamster, Guinea pig, cow, pig, goat, sheep, horse, and primate.

51. (Withdrawn) The non-human living organism of claim 50, wherein said organism is a mouse.

52. (Withdrawn) The non-human living organism of claim 50, wherein the organism is a yeast.

53. (Previously Presented) The DNA molecule of claim 16, wherein said protein is selected from the group consisting of a reporter protein and a selection marker.

54. (Currently Amended) An isolated nucleic acid comprising ~~the isolated a DNA of claim 1 further flanked by additional~~ SSRTS molecule comprising at least a sequence A flanked by at least site specific recombinase targeting sequences (SSRTS) L1, and at least a

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sequence B flanked by at least site specific recombinase targeting sequences (SSRTS) L2, said SSRTS L1 and SSRTS L2 being unable to recombine with one another, wherein:

(i) sequences L1 are in an orientation opposite to one another, wherein said sequences point towards each other or point away from each other,

(ii) sequences L2 are in an orientation opposite to one another, wherein said sequences point towards each other or point away from each other,

(iii) the order of SSRTS sequences in said DNA molecule is 5'-L1-L2-sequence A-sequence B-L1-L2-3'; and

(iv) said DNA molecule comprising 5'-L1-L2-sequence A-sequence B-L1-L2-3' is further flanked by additional site specific recombinase targeting sequences (SSRTS) L3, said SSRTS L3 being unable to recombine with said SSRTS L1 or SSRTS L2.